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PRELIMINARY COMMUNICATIONS

THE BILIARY EXCRETION OF BUCOLOME IN THE RAT : A POSSIBLE CAUSE FOR CHOLERESIS

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Bucolome (BC, 1-cyclohexyl-5-n-butyl-2,4,6-trioxoperhydropyrimidine), a nonsteroid antiinflammatory drug [1], is known to be a potent choleretic. It increases canalicular bile flow in rats, guinea pigs and dogs. Although it enhances the biliary excretion of endogenous bile salts in rats, the bile flow rate does not correlate with the bile salt excretion rate [2]. In rats or dogs in which the bile salt pool was depleted by an external biliary fistula, BC still caused a significant choleresis without increasing the bile salt excretion rate [2,3]. Thus, the basic nature of this choleresis is classified as bile salt independent. Furthermore, BC significantly enhanced the biliary excretion of ouabain in the rat [4], while all other anionic choleretics that have been tested are reported to reduce or leave ouabain excretion unchanged [5,6]. The mechanism of BC induced choleresis has not been elucidated. The possibility that BC or its metabolite(s) excreted into the bile might induce osmotic choleresis has been considered to be unlikely, since the amount of biliary excretion of BC or its metabolite(s) has been reported to be very small [7]. However, the data in the literature regarding the biliary excretion of BC was not sufficient to exclude this possibility. The authors examined the biliary excretion of this drug in the rat by using newly sysnthetized ^{14}C-labeled BC.

Materials and Methods ^{14}C-labeled BC was synthetized by one of the authors (TU) from ^{14}C-cyanate (purchased from RCC, Amersham), the details of which will be published elsewhere. In brief, cyclohexylurea which was made from cyclohexylamine and potassium cyanate was reacted with diethyl butylmalonate [1]. This crude product was purified by using column chromatography (Sephadex LH-20, solvent ethanol). The sodium salt of BC was made from free BC by the following procedure. Both radioactive BC, and unlabeled

BC (supplied by Takeda Chemical Industries, Osaka, Japan) were mixed separatly with equivalent metalic sodium in ethanol. After evaporation of solvent, the residue was recrystalized from ethanol-ethylacetate. The examination of the purified product of the sodium salt of labeled BC using thin layer chromatography (solvent, benzene : ethylether : acetate : methanol = 60:30:9:1, Silica Gel G) showed one major peak (Fig. 2,A) and one minor peak (Fig. 2,B). The Rf value of the major peak A was identical to that of the unlabeled BC preparation. The minor peak (B) corresponded to a hydroxylated BC described previously [7], which was probably produced during the thin layer chromatography process. The storage of the preparation for a month did not increase the amount of this hydroxylated BC.

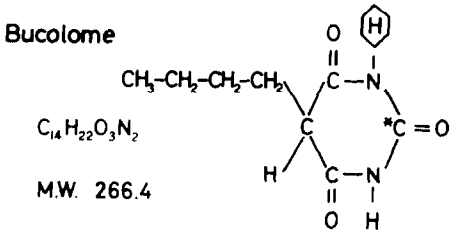

Fig. 1

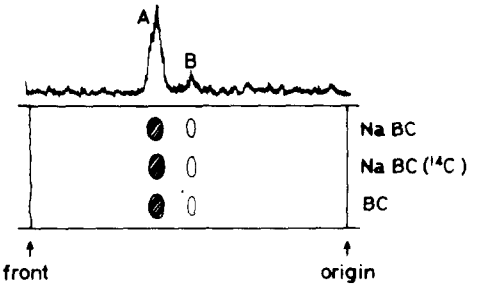

Fig. 2

Fig. 1. ^{14}C-labeled BC.
Fig. 2. Radiochromatogram of the sodium salt of ^{14}C-BC and thin layer chromatograms of different BC preparations. (from top to bottom, unlabeled sodium salt of BC, final preparation of sodium salt of labeled BC, and unlabeled BC)

Ten-week-old Wistar male rats (250g, body weight on the average) were anesthetized with pentobarbitone sodium (4.5mg/100g i.p.). In one group of rats, radioactive BC mixed with carrier BC in the form of a sodium salt in a saline solution was administered intraperitoneally (20mg/100g body weight) before opening the abdominal wall, approximately 0.5 μCi of the isotope being given to each rat. Five min later, the abdominal incision was made and the common bile duct was cannulated with PE-10 tubing. The abdomen was then closed. Bile collection began 15 min after BC administration and 7 successive bile samples were collected every 15 min. In another group of rats, the common bile duct was cannulated before BC injection. After administering BC (10mg/100g body weight, i.p.), bile was collected every 15 min for 2 hr. In both groups, arterial blood samples were taken at various time intervals through a femoral artery previously cannulated with PE-10 tubing. Physiologic saline was continuously infused through a femoral vein to compensate for the

loss of water by bile collection. Fifty μl of plasma or 200 μl of bile were mixed with 5 ml of scintillator (Aquasol-2, NEN. Boston, USA) in a mini vial. The radioactivity was counted in a liquid scintillation counter by using an automatic external standardization for the quenching correction.

Results and Discussion Fig. 3 shows the plasma BC levels and a cumulative biliary excretion of BC or its metabolite(s) as determined from the radioactivities of plasma and bile. The total biliary excretion of BC expressed as a percent of the administered dose was 24.5 ± 4.0 (n=3, mean ± SD) in rats given 10mg/100g of BC for 2 hr and 19.0 ± 4.0 (n=3) in rats given 20mg/100g from 15 min to 2 hr after BC administration. Fig. 4 shows the relationship between the bile flow rate and the biliary excretion rate of BC or its metabolite(s) as calculated from the radioactivity of the bile assuming all radioactivities in the bile have the same molar specific activity as the parent compound. A significant linear relationship was observed between the bile flow rate and the excretion rate of BC, 27 μl of bile being produced for each umole of BC excretion.

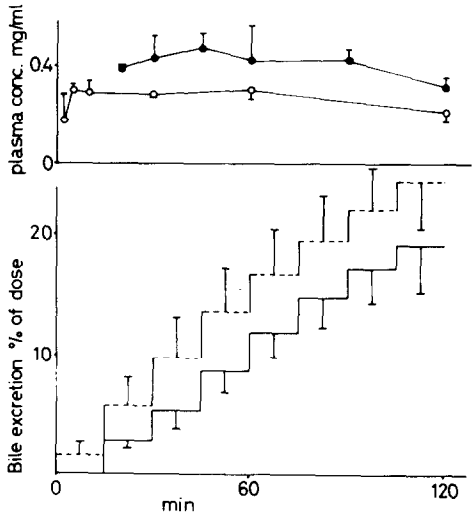

Fig. 3

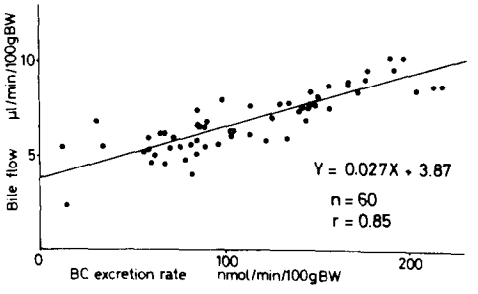

Fig. 4

Fig. 3. Plasma levels and cumulative biliary excretion of BC as determined by radioactive BC. Solid lines indicate the study on rats given 20mg/100g body weight of BC 15 min before bile collection. Dashed lines indicate the study on rats given 10mg/100g of BC. Each value is the mean of three rat experiments. A vertical bar indicates 1 SD.

Fig. 4. Relationship between the bile flow rate and the biliary excretion rate of BC. Least square regression analysis shows the relation, Y = 0.027X + 3.87.

In the past literature, biliary excretion of BC was reported as 2 percent of the dose for the first 8 hr in the rabbit [7]. In rats, no comparable information is available, but fecal excretion was reported to be 6 percent in the first 96 hr [8]. The plasma half life of BC was also stated to be 7 hr in the monkey, 14 hr in the rat and 17 hr in the rabbit [7]. On the basis of this evidence, the biliary excretion of BC could not be considered a major cause for its choleretic activity. Contrary to this anticipation, the present data indicate that, at least in rats, BC excretion in bile could be a major cause of choleresis, possibly due to the osmotic force of the excreted BC. The value of 27 μl of bile produced by 1 μmole of BC is two to three times higher than taurocholate [9,10] and even higher than dehydrocholate (18μl/umole) [11] or iodipamide (24μl/umole) [12]. The absence of a significant correlation between bile flow rate and bile salt excretion rate in BC administered rats reported previously [2] might be well explained by this efficient biliary excretion of BC (and/or its metabolite(s)) and its very potent choleretic activity.

The change in plasma BC levels observed in the present study was very slow, which agrees with previous studies reporting very long plasma half lives [7,8]. However it should be noted that the highest plasma level of BC on the average was 0.47mg/ml for rats given 20mg/100g, and 0.3mg/ml for rats given 10mg/100g respectively. This means that less than 10 percent of the injected dose appeared in the plasma. In one rat where BC was given iv (20mg/100g), the plasma level of BC was approximately 0.7mg/ml at 2 and 5 min after injection, the biliary recovery for 2 hr being 24.1 percent. It is possible that in animals without a bile fistula the enterohepatic circulation of BC maintains the plasma level of BC higher and longer than that observed in the present study. Although the enterohepatic circulation was suggested unlikely as a route for BC metabolism [7], only this enterohepatic circulation could reconcile the data given in the present study with a past report indicating that 72 percent of BC was excreted in the urine in the rat in 96 hr, while only 6 percent in the feces [8].

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